Vitamin B-12, Folic Acid, and Growth in 6- to 30-Month-Old Children: A Randomized Controlled Trial

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abstract

BACKGROUND: Folate and vitamin B-12 are important for growth. Many children in low- and middle-income countries have inadequate intakes of these nutrients.

METHODS: We undertook a randomized, placebo controlled double-blind trial in 1000 North Indian children, 6 to 35 months of age, providing twice the recommended daily allowance of folic acid and/or vitamin B-12, or placebo, daily for 6 months. By using a factorial design, we allocated children in a 1:1:1:1 ratio in blocks of 16. We measured the effect of giving vitamin B-12, folic acid, or the combination of both on linear and ponderal growth. We also identified predictors for growth in multiple linear regression models and effect modifiers for the effect of folic acid or vitamin B-12 supplementation on growth.

RESULTS: The overall effect of either of the vitamins was significant only for weight; children who received vitamin B-12 increased their mean weight-for-age *z* scores by 0.07 (95% confidence interval: 0.01 to 0.13). Weight-for-age *z* scores and height-for-age *z* scores increased significantly after vitamin B-12 supplementation in wasted, underweight, and stunted children. These subgrouping variables significantly modified the effect of vitamin B-12 on growth. Vitamin B-12 status at baseline predicted linear and ponderal growth in children not receiving vitamin B-12 supplements but not in those who did (*P*-interaction $< .001$).

CONCLUSIONS: We provide evidence that poor vitamin B-12 status contributes to poor growth. We recommend studies with larger doses and longer follow-up to confirm our findings.

WHAT'S KNOWN ON THIS SUBJECT: Micronutrient deficiencies, including deficiencies of vitamin B-12 and folate, are common worldwide and may be a contributing factor to the estimated 165 million stunted children.

WHAT THIS STUDY ADDS: Routine supplementation of vitamin B-12 improved linear and ponderal growth in subgroups of young Indian children. We provide evidence that vitamin B-12 deficiency is a contributor to poor growth in low- and middle-income countries.

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Dr Strand conceptualized and designed the study, analyzed data, drafted the first version of the manuscript, and had primary responsibility for the final content; Dr Taneja conceptualized and designed the study, supervised data management, analyzed data, and reviewed the manuscript; Dr Kumar supervised data collection and contributed to the study design and manuscript preparation; Dr Manger supervised data collection, contributed to study procedures, and reviewed the manuscript; Drs Refsum and Bhandari conceptualized and designed the study and reviewed the manuscript; Dr Yajnik supervised analysis of plasma specimens and interpretation of the results in addition to critically reviewing the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

This trial has been registered at www.clinicaltrials.gov (identifier NCT00717730) and at www.ctri.nic.in (identifier CTRI/2010/091/001090).

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Deficiencies of folate and vitamin B-12 are often part of general malnutrition and may contribute to poor growth and excess morbidity in children in many low- and middle-income countries (LMICs).[1–3] The main source of vitamin B-12 is animal-derived foods, which are expensive and for cultural and religious reasons often not eaten at all.[1] In addition to breast milk, the best sources of folates are dark-green vegetables and legumes.[2] It is likely that these foods are inaccessible or only eaten in small amounts by many urban poor. For example, a study in adult slum dwellers in New Delhi, India, found that 93% had folate intakes below the Indian recommended daily allowances (RDAs).[4] Many children in LMICs have suboptimal or poor status of either or both of these nutrients.[5] However, to what extent this situation has any consequences for their health and growth is not clear.

Folates and vitamin B-12 share metabolic pathways and are important for DNA and protein synthesis and therefore cell growth and differentiation.[6] Poor intake of either may accordingly be a contributing factor to poor growth in the estimated 165 million stunted children.[7]

Using a factorial design, we conducted a randomized placebo-controlled trial of folic acid and vitamin B-12 to measure whether daily administration of either or both of these nutrients improved growth in 1000 North Indian children aged 6 to 35 months.

METHODS

Study Population

This was a randomized, placebo-controlled double-blind trial in 1000 North Indian children aged 6 to 35 months in which the main objective was to measure the effect of giving folate and/or vitamin B-12 daily for 6 months on diarrhea and respiratory infections. The main objective of this report, and a secondary objective of the study, was to measure to what extent the vitamins improved linear and ponderal growth expressed as change in length (cm), weight (kg), and *z* scores (weight-for-age [WAZ] and height-for-age [HAZ]) from baseline until the end of the study. The study was implemented in the Tigri and Dakshinpuri areas in New Delhi, India. These neighborhoods comprise 300 000 inhabitants residing in 60 000 dwellings. Details of the study setting and the effect of the intervention on morbidity has been published previously.[8]

A door-to-door survey was conducted to identify children of either gender. Families consenting and not moving away over the next 6 months were considered for enrollment. Enrollment commenced in January 2010, and the last child completed the study in September 2011. Children with severe systemic illness requiring hospitalization, severe acute malnutrition (weight-for-height *z* score < -3), or severe anemia (hemoglobin <7 g/dL) and those taking folic acid and/or vitamin B-12 supplements were excluded. Children with anemia or with acute infections that required medical treatment were enrolled after recovery if eligible.

Randomization and Masking

Children were randomly assigned to 1 of 4 treatment groups: (1) placebo (n = 249), (2) twice the RDA of vitamin B-12 (n = 252), (3) twice the RDA of folic acid (n = 249), or (4) a combination of both vitamin B-12 and folic acid (n = 250). Children were assigned in a 1:1:1:1 ratio, in blocks of 16, by using a factorial design. The randomization scheme was generated by using Stata, version 10 (StataCorp, College Station, TX), by a scientist otherwise not involved with the trial. The vehicle for the vitamins and the placebo was a lipid-based paste provided in jars prelabeled with the subject's identification number and no indication of study group. The subject identification number (1–1000) was the only indication that could link the paste to the study group. The placebo and the vitamin supplements were identical in appearance and taste, and the allocation was masked to participants as well as the study team throughout the data collection period. The paste was prepared by Nutriset Ltd (Malaunay, France).

Children were supplemented with 1 spoonful (5 g) of the paste if they were 6 to 11 months old and with 2 spoonfuls (10 g) if they were older than 12 months. The paste was administered daily by a field-worker for 6 months at home except on Sundays or other public holidays when the caregiver administered the paste. The supplements were packaged in jars of 330 g; this supply was adequate for 1 month (for children aged >12 months) and for 2 months for children aged 6 to 11 months. Each 10 g of supplement contained 54.1 kcal of total energy, 0.71 g of protein, and 3.31 g of fat. In addition, for the groups who were randomly assigned to receive folic acid, the supplement also contained 150 μg of folic acid and for the vitamin B-12 groups it also contained 1.8 μg of vitamin B-12.

Enrolled children were visited twice weekly for 6 months to ascertain information on diarrheal and respiratory morbidity by a separate team of field-workers in addition to those who undertook the daily visits to dispense the supplements. Sick children who were referred or came spontaneously to the study clinic were treated according to World Health Organization guidelines for integrated management of neonatal and childhood illness.[9] Measurements of weight and length were taken at baseline and at the end of follow-up. Each measurement was performed twice. Weight was measured to the nearest 50 g by using Digitron scales (Digitron, New Delhi, India). Length was measured by using locally

manufactured infantometers to the nearest 0.1 cm. Additional details about the intervention, methods, and results on common infections are provided in the publication reporting the primary objective of the study.[8]

Ethics

The ethics committees of the Society for Essential Health Action and Training, the Society for Applied Studies, Christian Medical College, and the Norwegian Regional Committee for Medical and Health Research Ethics approved the study.

Sample Size

Trial size was calculated by using assumptions from a previous study in an adjacent slum.[10] This study was powered to assess an effect of the interventions on the incidence of infections, and the sample size requirements for measuring an effect on growth was lower. Expected differences in changes in *z* scores (WAZ and HAZ) from baseline until the end of the study of 0.2 and 0.3 required group sizes of 190 and 85, respectively. In these calculations, we assumed an SD of 0.6, a power of 90%, and an α of 0.05.

Laboratory Analysis

Blood samples were obtained at baseline for all children and at the end of the 26-week supplementation period in a subsample of 16 randomly selected blocks (256 children). Blood was collected into evacuated tubes containing EDTA and centrifuged at ~450 g at room temperature for 10 minutes, separated and transferred into storage vials, and stored at −20°C before analysis. Plasma total homocysteine (tHcy), a marker of folate and vitamin B-12 status,[11,12] was analyzed by using commercial kits (Abbott Laboratories, Abbott Park, IL).[13] Plasma concentrations of vitamin B-12 and folate were determined by microbiological assays by using a chloramphenicol-resistant strain of *Lactobacillus casei* and a colistin sulfate–resistant strain of *Lactobacillus leichmannii*, respectively.[14]

Standardization and Quality Control

Standardization exercises were conducted to achieve agreement between workers for weight and length measurements. Subsequent to practice sessions to measure weight and length, workers participated in exercises in which 10 sets of 10 children were measured twice. Standardization exercises for weight ended when all workers obtained identical readings in both their measurements and were in perfect agreement with the group mean (ie, arithmetic mean of all observations of the workers for a set of 10 children). A difference of ±0.5 cm between the reading of the worker and the group mean was considered acceptable for length. Weighing scales were calibrated daily against a known standard weight and an infantometer with standard steel rods.

Statistical Analysis

The forms for the study were designed in Visual Basic.net with range and consistency checks incorporated. Double data entry by 2 data clerks followed by validation was completed within 72 hours. In the baseline table (Table 1), continuous variables were reported as means or medians as appropriate and categorical variables were reported as proportions. *z* Scores for weight-for-height, WAZ, and HAZ were calculated by using World Health Organization reference values.[15] We compared the absolute change in centimeters and kilograms and change in *z* scores from baseline to the end of the study by using multiple linear regression analyses. Dichotomous variables that indicated whether vitamin B-12 or folic acid was given were included in the multiple models simultaneously. In these analyses, we compared those who received folic acid (with or without vitamin B-12; n = 499) with those who did not receive folic acid (with or without vitamin B-12; n = 501) and those who received vitamin B-12 (n = 502) with those who received placebo (n = 498). We also compared each of the groups receiving B vitamins (folic acid, vitamin B-12, and folic acid with vitamin B-12) with the group who only received placebo. In these

TABLE 1 Baseline Characteristics of Children Aged 6 to 30 Months Randomly Assigned to Receive Placebo, Folate, and/or Vitamin B-12 for 6 Months

Characteristics	Placebo (n = 249)	Vitamin B-12 (n = 252)	Folate (n = 249)	Vitamin B-12 + Folate (n = 250)
Age at enrollment, mean (SD), mo	16.4 (7.1)	15.9 (6.9)	16.2 (7.3)	15.9 (7.0)
Proportion of infants, *n* (%)	80 (32.1)	80 (31.8)	81 (32.5)	80 (32.0)
Boys, *n* (%)	135 (54.2)	115 (45.6)	125 (50.2)	132 (52.8)
Girls, *n* (%)	114 (45.8)	137 (54.4)	124 (49.8)	118 (47.2)
Breastfed, *n* (%)	181 (72.7)	188 (74.6)	172 (69.1)	182 (72.8)
Literate mother, *n* (%)	185 (74.3)	183 (72.6)	196 (78.7)	192 (76.8)
Annual family income, median (IQR), 1000 rupees	84 (53–144)	72 (48–120)	72 (60–156)	76 (60–140)
z Scores, mean (SD)				
WHZ	−0.90 (0.89)	−0.93 (0.94)	−0.73 (1.0)	−0.90 (0.89)
HAZ	−1.64 (1.14)	−1.68 (1.25)	−1.55 (1.25)	−1.54 (1.17)
WAZ	−1.53 (0.98)	−1.58 (1.09)	−1.36 (1.13)	−1.48 (1.04)
Wasted				
< −2 WHZ	28 (11.2)	32 (12.7)	23 (9.2)	22 (8.8)
Stunted				
< −2 HAZ	97 (39.0)	93 (36.9)	91 (36.6)	84 (33.6)
Underweight				
< −2 WAZ	80 (32.1)	81 (32.1)	75 (30.1)	73 (29.2)
Hemoglobin concentration <11 g/dL, *n* (%)	181 (72.7)	170 (67.5)	161 (64.7)	184 (73.6)

IQR, interquartile range; WHZ, weight-for-height *z* score.

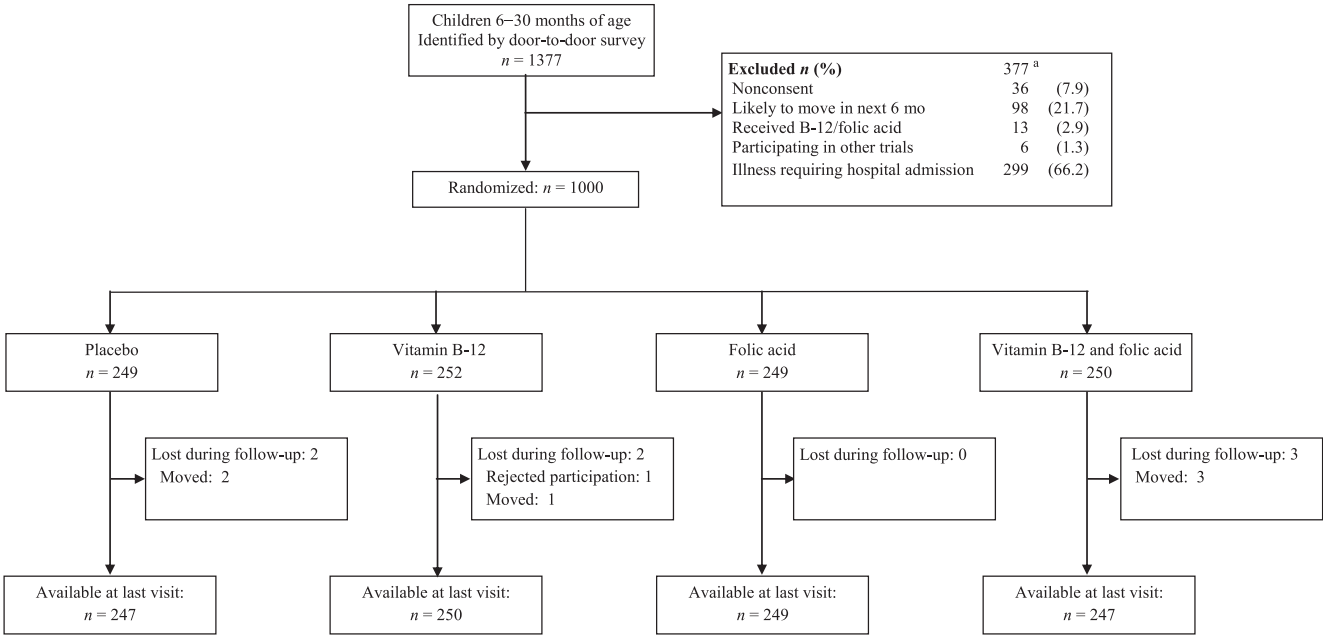

FIGURE 1

Trial profile of a randomized placebo-controlled trial on the effect of folic acid and/or vitamin B-12 administration in 6- to 30-month-old North Indian children. [a]For 60 children, there were multiple reasons for exclusion: fever (70), acute lower respiratory infections (ALRI) (92), hemoglobin (73), diarrhea (12), vomiting (6), lethargy (1), dysentery (3), dehydration (3), hepatitis (1), extensive skin infection (1), hydrocephalus (2), hepatosplenomegaly (1), coronary heart disease (1), nephrotic syndrome (1), wasting (7), mastoid (1), length-for-age *z* score < -3 SDs (22), and cause unknown (2).

analyses each treatment arm contained ~250 children. We additionally explored the effects of giving vitamin B-12 or folic acid in various predefined subgroups. We undertook these subgroup analyses unadjusted and adjusted for baseline age, breastfeeding status, gender, as well as HAZ and WAZ. We also included interaction terms to measure whether the effects of folic acid or vitamin B-12 were significantly different between the subgroups as well as to measure the interaction between folic acid and vitamin B-12 supplementation. Only 1-way interactions were included in the statistical models. We also identified predictors for change in WAZ and HAZ in multiple linear regression models. The variables listed in Table 1 were initially assessed in crude models; those that were significant at a 0.2 level were included in a multiple model. Each of the variables that were not associated with the outcome in the crude models were then included one at a time and retained if significant. We also assessed the interaction between age (continuous) and breastfeeding (dichotomous), baseline vitamin B-12 concentration (continuous) and vitamin B-12 supplementation (dichotomous), and baseline folate concentration (continuous) and folic acid supplementation. Relative differences between biochemical markers were calculated by linear regression of the log-transformed values of the change in plasma vitamin B-12, folate, or tHcy from baseline to the end of the study. For these biochemical outcomes, we also compared those who received folic acid (with or without vitamin B-12) with those who did not receive folic acid (with or without vitamin B-12) and those who received vitamin B-12 with those who received placebo. Statistical analyses were performed with Stata, version 13. All analyses for the effects of the interventions

TABLE 2 Folate and Vitamin B-12 Status at Baseline and the End of Study in North Indian Children Aged 6 to 30 Months Randomly Assigned to Receive Placebo, Folic Acid, and/or Vitamin B-12 Daily for 6 Months

	Placebo	Vitamin B-12	Folic Acid	Vitamin B-12 + Folic Acid
Baseline				
n	249	252	249	250
Plasma vitamin B-12, pmol/L	266 (165–381)	253.5 (172–404)	265 (176–425)	277.0 (187–416)
Plasma folate, nmol/L	11.4 (6.8–19.5)	10.6 (6.6–20.8)	11.5 (6.7–20.7)	11.1 (6.3–20.5)
Plasma tHcy, μmol/L	11.9 (9.1–16.9)	11.2 (8.9–16.1)	11.5 (8.7–15.3)	11.6 (8.9–15.4)
End of study				
n	64	65	67	66
Plasma vitamin B-12, pmol/L	318 (191–404)	381 (282–567)	317 (248–492)	455 (307–605)
Plasma folate, nmol/L	15.3 (9.8–21.5)	13.3 (8.5–18)	46.5 (27.9–64.3)	47.7 (30.7–67)
Plasma tHcy, μmol/L	10.7 (8.5–13.9)	8.5 (6.8–10.5)	7.3 (6.1–9.6)	6.8 (5.7–9.4)

Data are presented as medians (interquartile ranges) unless otherwise indicated.

(vitamin B-12 and/or folic acid) were performed by intention-to-treat.

RESULTS

We identified 1377 children aged 6 to 30 months and randomly assigned 1000 children. Figure 1 shows the flow of the participants through the study. Baseline features were comparable between the groups. Most of the children were breastfed. Forty-two percent of the children were stunted, 34% were underweight, and 10.5% were wasted. One-third had a vitamin B-12 concentration ≤200 pmol/L, almost one-third had a baseline folate concentration <7.5 nmol/L, and elevated tHcy was seen in more than half of the children. Approximately 70% of the children were anemic. These baseline characteristics are shown in Table 1. Among the randomly assigned children, ~99% were available for the last scheduled visit, and there were only 7 children who withdrew before the scheduled end of follow-up. Compliance was good, with 96% of the scheduled doses reportedly ingested, which is reflected in the change in the concentrations of folate, vitamin B-12, and tHcy from baseline to the end of follow-up. The geometric mean vitamin B-12 concentration was 1.28 (95% confidence interval [CI]: 1.14–1.44) times higher in those who were given supplements containing vitamin B-12 compared with those who were not. Similarly the geometric mean folate concentration was 3.14 (95% CI: 2.56 to 3.86) times higher in those who were given folic acid. Both vitamin B-12 and folic acid supplementation resulted in a decreased tHcy concentration with geometric mean ratios of 0.78 (95% CI: 0.72 to 0.83) and 0.83 (95% CI: 0.77 to 0.89), respectively. The baseline and end-of-study median (interquartile range) concentrations of these markers by study group are shown in Table 2.

The effects of giving folic acid or vitamin B-12 on weight, length, and growth indices are given in Table 3. When comparing all children who received vitamin B-12 with those who did not, the effect on growth indices was small and was statistically significant only for WAZ. Adjusting for important baseline factors did not alter these estimates. Compared with placebo, the administration of vitamin B-12 in combination with folic acid increased mean weight and length by 120 g (95% CI: 3 to 210 g) and by 0.35 cm (95% CI: 0.0 to 0.66 cm), respectively.

TABLE 3 Unadjusted Changes in Anthropometric Values in North Indian Children Given Placebo, Vitamin B-12, or Folic Acid for 6 Months

	Placebo Group	Supplement Group	Mean Difference (95% CI)
Vitamin B-12			
n	502	498	
Weight, kg	1.03 (0.57)	1.09 (0.54)	0.06 (−0.01 to 0.13)
Length/height, cm	5.33 (1.93)	5.40 (1.83)	0.07 (−0.16 to 0.31)
z Scores			
Length-for-age	−0.01 (0.58)	0.00 (0.56)	0.01 (−0.06 to 0.09)
WAZ	−0.05 (0.51)	0.02 (0.52)	0.07 (0.01 to 0.13)*
Weight-for-length	−0.11 (0.67)	−0.03 (0.69)	0.08 (−0.01 to 0.17)
Folic acid			
n	499	501	
Weight, kg	1.03 (0.51)	1.09 (0.60)	0.05 (−0.02 to 0.12)
Length/height, cm	5.26 (1.67)	5.46 (2.07)	0.20 (−0.03 to 0.44)
z Scores			
Length-for-age	−0.19 (0.52)	−0.14 (0.69)	0.04 (−0.03 to 0.11)
WAZ	−0.12 (0.50)	−0.09 (0.55)	0.03 (−0.03 to 0.09)
Weight-for-length	−0.08 (0.67)	−0.07 (0.69)	0.00 (−0.08 to 0.09)

Data are presented as means (SDs) unless otherwise indicated. Unadjusted changes in anthropometric variables during the intervention are shown. The interaction between vitamin B-12 and folic acid supplementation was not significant for any of the outcomes listed in this table. *$P < .05$.

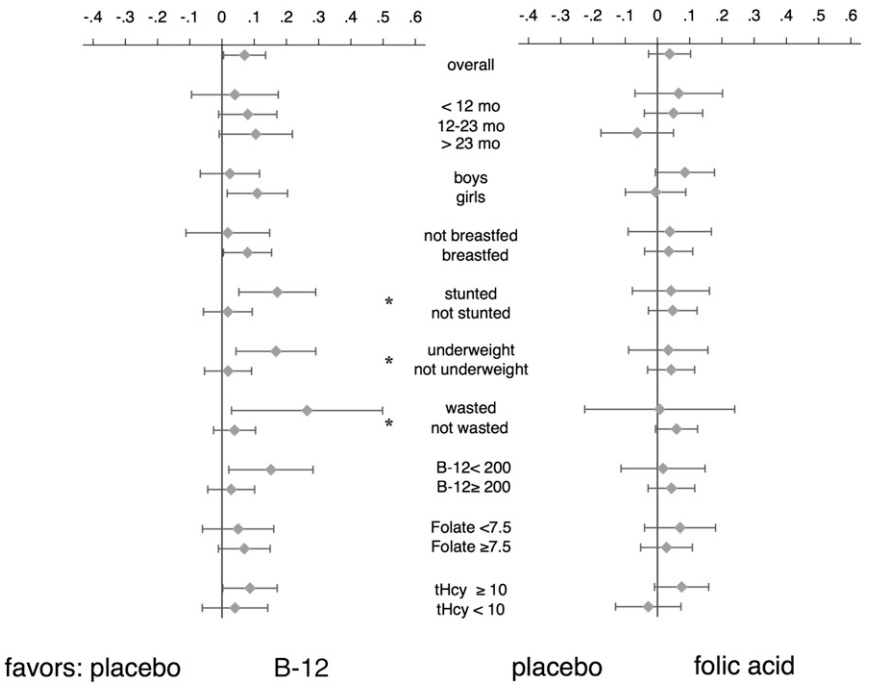

FIGURE 2
Effects of folic acid or vitamin B-12 supplementation on WAZ in North Indian children 6 to 30 months of age in various subgroups on the basis of baseline characteristics. Horizontal bars show 95% CIs of the mean difference in change in WAZ. *Significant interaction term between the subgrouping variable and the intervention, $P < .05$.

Vitamin B-12 supplementation significantly improved HAZ and WAZ in stunted, underweight, and wasted children. The interaction terms for vitamin B-12 and these subgrouping variables were all significant at a P value $<.05$. Vitamin B-12 administration also improved WAZ in children who had a vitamin B-12 concentration <200 pmol/L; however, low vitamin B-12 did not significantly modify the effect of vitamin B-12 on WAZ. Low folate status, defined as folate <7.5 nmol/L, significantly modified the effect of folic acid on HAZ: children who had low levels of folate at baseline significantly benefited from folic acid supplementation for 6 months. The subgroup effects were not substantially altered when adjusting for age, gender, breastfeeding status (whether a child was breastfed at the time of enrollment), HAZ, and WAZ at baseline (Figs 2 and 3). There were no significant interactions between folic acid administration and vitamin B-12 administration on any of the outcomes.

The associations between various baseline features, vitamin B-12, or folic acid administration and growth are shown in Table 4. Vitamin B-12 concentration at baseline predicted both WAZ and HAZ changes but only in children who were not supplemented with vitamin B-12 (P-interaction $< .001$) for both outcomes.

DISCUSSION

In this study we provide evidence that deficiencies of vitamin B-12 and folate contribute to poor linear and ponderal growth. Subgroups of children randomly assigned to either of these nutrients improved their HAZ and WAZ compared with placebo, and vitamin B-12 status at baseline was positively associated with growth over the subsequent 6 months in children not receiving vitamin B-12.

We are not aware of any other randomized placebo-controlled trial that has measured the effect of these vitamins on growth. The compliance was excellent and reflected in the plasma concentrations of vitamin B-12, folate, and tHcy at the end of the study. The overall effect was small and only significant for vitamin B-12 and on WAZ when the full sample of children was included (Table 3, Fig 2). However, our subgroup analyses revealed that vitamin B-12 supplementation was significantly beneficial in children with impaired growth (ie, those who were defined as being too short and/or too thin at baseline). Furthermore, wasting, stunting, and underweight were the only subgroups in whom there was a significant effect of giving vitamin B-12 on both HAZ and WAZ (Figs 2 and 3).

Other subgroups of children also benefited from folic acid or vitamin B-12 supplementation (Figs 2 and 3). However, except for poor folate status, the interaction terms between these subgrouping variables and vitamin B-12 or folic acid administration on change in HAZ or WAZ were not statistically significant. Folate status at baseline was the only subgrouping variable that significantly modified the effect of folic acid supplementation on growth; children with poor folate status had improved linear growth when given folate supplementation, whereas children with apparently adequate folate status did not.

Vitamin B-12 is required for the folate-dependent enzyme methionine synthase, which is necessary for the synthesis of methionine from homocysteine. Methionine in its activated form, *S*-adenosylmethionine, is the major methyl group donor used in human methylation reactions, including methylation of DNA and RNA. Deficiencies of vitamin B-12 and folate therefore have similar consequences on cellular division and differentiation, and both result in elevated tHcy concentrations.[11] In

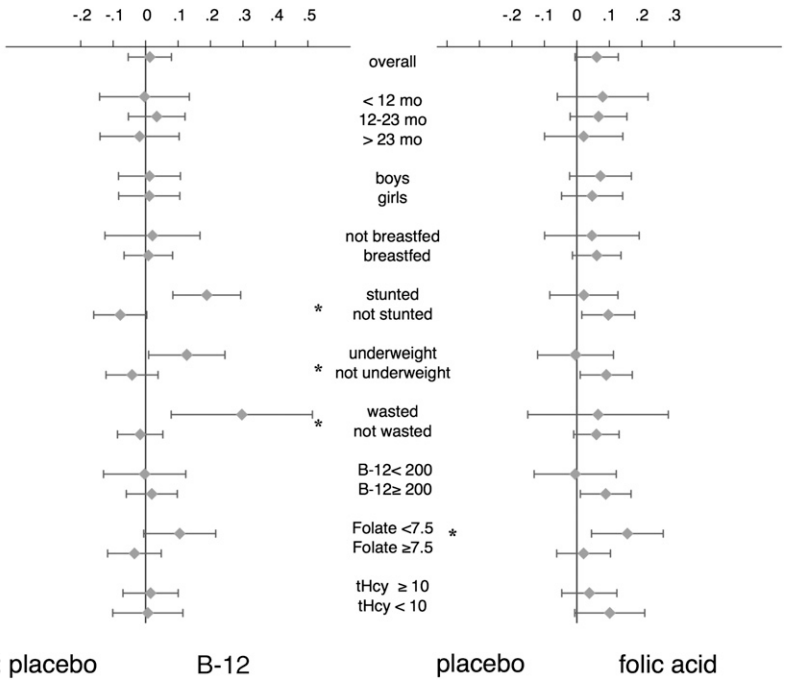

FIGURE 3

Effects of folic acid or vitamin B-12 supplementation on HAZ in North Indian children 6 to 30 months of age in various subgroups on the basis of baseline characteristics. Horizontal bars show 95% CIs of the mean difference in change in HAZ. *Significant interaction term between the subgrouping variable and the intervention, $P < .05$.

TABLE 4 Baseline Predictors for Changes in HAZ and WAZ Over 6 Months in North Indian Children Aged 6 to 30 Months

Model	Coefficient[a]	95% CI	*P*
Change in length-for-age *z* scores from baseline until end of study			
Gender (reference: girls)	0.06	(−0.01 to 0.12)	.085
Years of schooling (father)	0.01	(0.002 to 0.02)	.017
Family income (log rupees)	0.05	(0.003 to 0.11)	.038
Vitamin B-12 group (B-12 given daily or not)[b]	0.95	(0.32 to 1.58)	.003
B-12 concentration (log pmol/L) in placebo group	0.21	(0.13 to 0.30)	<.001
B-12 concentration (log pmol/L) in vitamin B-12 group	0.05	(−0.03 to 0.13)	.226
Vitamin B-12 group × log B-12 concentration	−0.17	(−0.28 to −0.05)	.004
Age	0.01	(0.004 to 0.01)	<.001
WAZ	0.13	(0.07 to 0.18)	<.001
Length-for-age *z* score	−0.26	(−0.30 to −0.21)	<.001
Change in WAZ from baseline until end of study			
Gender (reference: girls)	0.05	(−0.01 to 0.11)	.087
Family income (log rupees)	0.07	(0.03 to 0.11)	.002
Breastfed[a]	0.46	(0.20 to 0.71)	<.001
Age in breastfed (months)	0.01	(0.00 to 0.01)	.025
Age in not breastfed (months)	−0.01	(−0.02 to −0.001)	.039
Breastfed × age	−0.02	(−0.03 to −0.01)	.005
Vitamin B-12 group (B12 given daily or not)[b]	0.84	(0.25 to 1.42)	.005
B-12 conc. (log pmol/L) in placebo group	0.15	(0.07 to 0.23)	<.001
B-12 conc. (log pmol/L) in B-12 group	0.01	(−0.07 to 0.08)	.833
Vitamin B-12 group × log B-12 concentration	−0.14	(−0.25 to −0.04)	.008
WAZ	−0.24	(−0.28 to −0.19)	<.001
HAZ	0.09	(0.04 to 0.13)	<.001

[a] Regression coefficients and 95% CIs were derived from multiple linear regression models.

[b] These coefficients are the differences in the intercepts between vitamin B-12 and placebo recipients or breastfed and nonbreastfed (ie, the hypothetical difference between the groups when plasma vitamin B-12 concentration is 0). These estimates should accordingly be interpreted with caution and have no practical value beyond being part of the regression model output.

this trial, both vitamin B-12 and folic acid administration resulted in substantial and significant reductions in mean plasma tHcy concentration, indicating that there are functional vitamin B-12 and folate deficiencies in this population that again can have clinical consequences such as slowed growth and development.[16]

There are several causes of impaired growth, including repeated infections and poor nutrition and the predictors presented in Table 4. Socioeconomic status (family income and schooling) predicted ponderal and linear growth, and WAZ at baseline was positively associated with linear growth over the next 6 months. Protein-energy malnutrition and various micronutrient deficiencies often coexist.[7] Malnourished (ie, growth-retarded) children are also those who are most likely to be deficient in other growth-limiting nutrients,[17] and it was among these that we found significant effects of vitamin B-12 administration. Concomitant deficiencies of other growth-limiting macro- and micronutrients, such as zinc,[18] may attenuate the effect of supplementation of any single growth-enhancing nutrient such as vitamin B-12. This interaction is also reflected in the results in our study in which supplementation of folic acid significantly improved linear growth only in children who had a cobalamin concentration >200 pmol/L and the effect of providing both vitamin B-12 and folic acid was higher than only providing vitamin B-12. However, it should be noted that the interaction terms between vitamin B-12 status and folic acid and vitamin B-12 supplementation and folic acid supplementation were not significant for any outcome. Preexisting malnutrition may imply a larger growth potential; however, as discussed above, malnutrition is associated with deficiencies of other growth-limiting nutrients, which may reduce the potential effect of vitamin B-12. Thus, the observed effect sizes in the subgroups of children who were wasted, stunted, or underweight could, in fact, be an underestimation of the real effect of vitamin B-12.

To estimate the potential effect of vitamin supplementation on growth it is important to ensure that a sufficiently high dose is given. The absorption of vitamin B-12 is complex, in which different parts of the gut are involved and bacterial overgrowth or other conditions may interfere with its absorption. Vitamin B-12 status is the most important predictor for plasma tHcy in this population.[3] We found a substantial and significant reduction in tHcy concentration after folic acid or vitamin B-12 supplementation. The excellent compliance was reflected in a threefold increase in plasma folate concentrations. However, the median tHcy concentration was still very high at the end of the study and the plasma vitamin B-12 concentration quite low despite a significant increase from baseline in the group of children receiving vitamin B-12. In our study the (geometric) mean vitamin B-12 concentration increased by ~28% compared with placebo. Other intervention studies with oral or parenteral vitamin B-12 have usually demonstrated at least a twofold increase in plasma cobalamin concentration compared with controls.[19–21] These studies also typically provided doses that were severalfold higher than the RDA and were able to show an even stronger effect on plasma tHcy concentrations. We also demonstrated that plasma cobalamin concentration was positively associated with linear and ponderal growth. The fact that supplementation of vitamin B-12 had a limited effect on plasma cobalamin levels in our study might be an indication that our doses were too low to result in any meaningful effects on growth.

Moreover, a study to measure the effect on growth could possibly be carried out for a period longer than 6 months, especially when measuring the effect on linear growth. The lack of a substantial and significant overall effect may accordingly be due to a combination of low doses and short exposure time. Future studies should consider higher doses and longer supplementation times.

In summary, we provide evidence that folate and vitamin B-12 are growth-limiting nutrients in this population. Despite a substantial increase in food security and improved health care in many LMICs such as in India, almost half of all children under the age of 5 are stunted, which is linked to serious health consequences,[7] including impaired brain development. Stunting is not only caused by lack of food it can also be caused by several other poverty-related factors and deficiencies of specific nutrients such as folate and vitamin B-12. We need studies that can estimate the relative importance of several modifiable risk factors for poor growth, including poor sanitation, clean water, immunization, proper and prompt treatment of infections, maternal education, and nutritional deficiencies. The degree to which suboptimal folate and vitamin B-12 status contributes to this complex scenario should be a prioritized research question.

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